

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

(12)

**EUROPEAN PATENT APPLICATION**

(21) Application number: 85810342.7

(51) Int. Cl.<sup>4</sup>: **A 61 K 37/02**  
**//C07K7/06**

(22) Date of filing: 24.07.85

(30) Priority: 02.08.84 GB 8419716  
02.08.84 GB 8419715

(43) Date of publication of application:  
05.02.86 Bulletin 86/6

(84) Designated Contracting States:  
AT BE CH DE FR GB IT LI LU NL SE

(71) Applicant: SANDOZ AG  
Lichtstrasse 35  
CH-4002 Basel(CH)

(84) Designated Contracting States:  
BE CH FR GB IT LI LU NL SE

(71) Applicant: SANDOZ-PATENT-GMBH  
Humboldtstrasse 3  
D-7850 Lörrach(DE)

(84) Designated Contracting States:  
DE

(71) Applicant: SANDOZ-ERFINDUNGEN  
Verwaltungsgesellschaft m.b.H.  
Brunner Strasse 59  
A-1235 Wien(AT)

(84) Designated Contracting States:  
AT

(72) Inventor: Borel, Jean-François  
Dornacherweg 4  
CH-4144 Arlesheim(CH)

(72) Inventor: Donatsch, Peter  
Herrenweg 34  
CH-4123 Allschwil(CH)

(72) Inventor: Hiestand, Peter  
Schönenbuchstrasse 13A  
CH-4123 Allschwil(CH)

(72) Inventor: Ryffel, Bernhard  
Schweizergasse 8  
CH-4054 Basel(CH)

(54) Novel pharmaceutical use of (NVA)2-and dihydro-(VAL)2-cyclosporine.

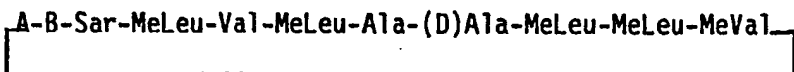
(57) Novel pharmaceutical use for the compounds (Nva)<sup>2</sup>-and dihydro-(Val)<sup>2</sup>-Cyclosporine, in particular their use in the treatment of autoimmune diseases selected from the group consisting of:

a) Uveitis; b) Juvenile diabetes type 1, c) Myasthenia gravis, d) Multiple sclerosis, e) Systemic lupus erythematosus, f) Haemolytic anaemia, and g) Glomerulonephritis.

Case 100-6412NOVEL PHARMACEUTICAL USE OF (NVA)<sup>2</sup>- AND DIHYDRO-  
-(VAL)<sup>2</sup>-CYCLOSPORINE

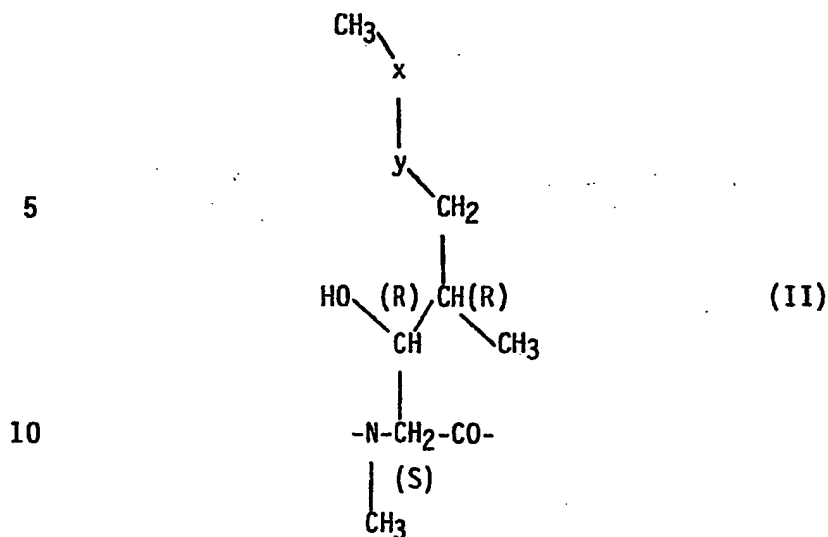
The present invention relates to a new use, in particular a new pharmaceutical use, for the compounds dihydrocyclosporin D and  
5 cyclosporin G, also known in accordance with current nomenclature and referred to throughout the present specification and claims as dihydro-(Val)<sup>2</sup>-Cyclosporine and (Nva)<sup>2</sup>-Cyclosporine respectively, as well as to pharmaceutical compositions comprising these compounds, or their use in the preparation of pharmaceutical com-  
10 positions for application in relation to said use.

Dihydro-(Val)<sup>2</sup>-Cyclosporine, which has the formula I



(I)

wherein A represents the residue of formula II



in which -x-y- is -CH<sub>2</sub>--CH<sub>2</sub>-, and B is -Val-, and (Nva<sup>2</sup>)-Cyclosporine, which has the formula II above, wherein A represents the  
 15 residue of formula II above in which -x-y- is -CH=CH-(trans), and B is Nva, are known and are described together with processes for their production as well as various pharmaceutical utilities, e.g. in US patent specifications nos. 4,220,641 and 4,288,431 respectively.

20 Chemically they belong to the distinct and now substantial class of natural and synthetic or semi-synthetic undecapeptides collectively designated as the cyclosporins [see for example US patent No. 4,117,118 (cyclosporin A or Cyclosporine); US patents Nos. 4,108,985 and 4,210,581 (cyclosporin C or (Thr)<sup>2</sup>-Cyclosporine,  
 25 and derivatives); as well as European patent publication No. 0,058,134 B1 and Helv. Chim. Acta. 65, Fasc. 5, pp. 1655 - 1677 (1982) disclosing yet further naturally occurring and synthetic or semi-synthetic cyclosporins including inter al. (Leu)<sup>10</sup>-Cyclosporine, [(D)Ser]<sup>8</sup>-Cyclosporine and (Val)<sup>11</sup>-Cyclosporine].

Of the cyclosporins the parent compound, cyclosporin A or Cyclosporine, has so far received the most attention. As disclosed in the aforementioned US patent No. 4,117,118 it possesses anti-inflammatory and anti-arthritic properties, as well as immunosuppressive properties which render the compound of especial utility in the prevention of rejection following organ transplant operations as well as in the treatment of auto-immune diseases.

The primary area of clinical investigation of Cyclosporine has been in its application to recipients of organ, e.g. heart, lung, combined heart-lung, liver, kidney, pancreatic, bone-marrow, skin and corneal transplants and, in particular, allogenic organ transplants. Cyclosporine is now commercially available for use in relation to organ transplant and a measure of its remarkable success is provided by the uncommonly wide attention the compound has received, not only in the scientific literature, but also in the lay press world-wide. At the same time, investigation of the applicability of Cyclosporine to the treatment of various auto-immune diseases has been intensive and reports of results in-vitro, in animal models and in clinical trials are wide-spread in the literature. The variety of auto-immune diseases is in fact very large. Though inevitably reported success for Cyclosporine has tended to vary in degree, e.g. from one auto-immune disease to another, broad utility is established and in some instances available results are sufficiently encouraging to warrant ongoing world-wide specialist attention.

Specific auto-immune diseases for which Cyclosporine treatment has been investigated or proposed include multiple sclerosis, Guillain-Barré syndrome, uveitis, myasthenia gravis, Heymann nephritis, Grave's disease, Hashimoto's thyroiditis, juvenile diabetes type I, systemic lupus erythematoses, aplastic anaemia,

pure red cell anaemia, idiopathic thrombocytopenia, polychondritis, scleroderma, Wegner granulomatosis, dermatomyositis, chronic active hepatitis, autoimmune male infertility, psoriasis and psoriatic arthritis, Steven-Johnson syndrome, idiopathic sprue, Chron's disease, sarcoidosis, glomerulonephritis, interstitial lung fibrosis and primary biliary cirrhosis.

As disclosed, e.g. in US patent specification No. 4,220,641, dihydro-(Val)<sup>2</sup>-Cyclosporine also possesses anti-inflammatory and anti-arthritic properties as well as immunosuppressive properties. With respect to immunosuppression, it has now been found that the specific immunosuppressive profile of dihydro-(Val)<sup>2</sup>-Cyclosporine differs from that of Cyclosporine in that it exerts markedly less influence on purely humoral immuno-response. Thus while dihydro-(Val)<sup>2</sup>-Cyclosporine, like Cyclosporine, may be anticipated to find application in the treatment of autoimmune disease, clinical utility would be primarily directed to the treatment of those particular conditions, e.g. as recited above, in which suppression of cellular rather than purely humoral response is critical. The present invention resides in part in the finding that dihydro-(Val)<sup>2</sup>-Cyclosporine is of surprising and unexpected advantageous utility in its application to two specific conditions of this latter type, namely a) Uveitis and b) Juvenile diabetes type I.

More especially it may be shown that in relation to the above specified auto-immune diseases dihydro-(Val)<sup>2</sup>-Cyclosporine exhibits particular and unexpected advantage in terms of e.g. level of activity and/or reduction or absence of undesirable side reactions in comparison with previously proposed therapy, e.g. employing Cyclosporine.

As disclosed e.g. in US patent specification No. 4,288,431, (Nva)<sup>2</sup>-Cyclosporine also possesses anti-inflammatory and anti-arthritic properties as well as immunosuppressive properties in test methods previously applied to Cyclosporine and to have the  
5 same utility profile and clinical utility as Cyclosporine.

(Nva)<sup>2</sup>-Cyclosporine like Cyclosporine may thus in its turn be anticipated to find application primarily in the prevention of transplant rejection as well as in the treatment of auto-immune disease. In further part the present invention resides in the  
10 finding that, while (Nva)<sup>2</sup>-Cyclosporine may be confirmed to possess the same general utility as Cyclosporine, i.e. in terms of its applicability to prevention or organ transplant rejection and to the treatment of auto-immune diseases as a broad class, its particular profile of activity, especially its particular  
15 profile in relation to immunosuppressive effect, render the compound surprisingly and unexpectedly advantageous in its application to certain specific diseases within the broad auto-immune disease group, namely:

a) Uveitis and b) Juvenile diabetes type I (i.e. the two specific  
20 diseases recited above in relation to utility of dihydro-(Val)<sup>2</sup>-Cyclosporine in accordance with the present invention) as well as: c) Myasthenia gravis, d) Multiple sclerosis, e) Systemic lupus erythematosus; f) Haemolytic anaemia, and g) Glomerulonephritis.

25 More especially it may be shown that in relation to the above specified auto-immune diseases (Nva)<sup>2</sup>-Cyclosporine exhibits particular and unexpected advantage in terms of e.g. level of activity and/or reduction or absence of undesirable side reactions in comparison with previously proposed therapy, e.g. employing  
30 Cyclosporine.

It is further to be noted that, while the compounds Cyclosporine, dihydro-(Val)<sup>2</sup>-Cyclosporine and (Nva)<sup>2</sup>-Cyclosporine are all of evidently very closely related chemical structure, their respective utilities in relation to the treatment of auto-immune disease, e.g. as determined in accordance with the findings of the present invention, while evidently paralleling one another, also exhibit crucial distinctions. In particular it is to be noted that while (Nva)<sup>2</sup>-Cyclosporine is now found to be of particular utility in the treatment of seven specific auto-immune disease types, dihydro-(Val)<sup>2</sup>-Cyclosporine is found to be of particular utility in two of these seven only. Thus in accordance with the invention structural similarity may not be seen as predictive of relative utility in relation to the subject invention.

15 The particular and advantageous utility of the compounds dihydro-(Val)<sup>2</sup>-Cyclosporine and (Nva)<sup>2</sup>-Cyclosporine in the treatment of the above specified auto-immune diseases can be demonstrated in recognised standard animal models as well as in clinical trials conducted for example as follows:

# 1. UVEITIS

## Modulation of Experimental Autoimmune Uveitis (EAV)

Testing is carried out in accordance with the general methodology described by Nussenblatt et al. in Arch. Ophthalmol. 100, 1146 - 1149 (1982). Groups of 6 to 10 Lewis (Q) rats weighing ca. 150 - 200 g are immunized with 30 µg of bovine S antigen emulsified (1:1 p.p.w.) in complete Freund's adjuvant enriched with 2.5 mg/mL Mycobacterium tuberculosis H 37 RA, by injection into the hind foot pad.

- 10 Test substance, i.e. dihydro-(Val)<sup>2</sup>-Cyclosporine or (Nva)<sup>2</sup>-Cyclosporine, is administered at a dosage of from 25 to 50 mg/kg/day i.m. administered on 7 consecutive days commencing 7 days after immunization. Control groups receive olive oil in place of test substance. The rats are killed on the 14th. day following
- 15 immunization and the eyes are removed immediately, fixed in formaldehyde, embedded in paraffin wax and stained with hematoxylin-eosin and PAS. Histopathologic evaluation is performed in masked fashion and inflammation graded on a scale of from 0 (no inflammation) to 4 (panophthalmitis). Selected cases are examined
- 20 by transmission and scanning electron microscopy. Eyes from animals exhibiting EAV show generalised inflammation of the retina and choroid with inflammatory cells enmeshed in a fibrous exudate occurring in the vitreous cavity, subretinal space and anterior chamber.
- 25 On administration of dihydro-(Val)<sup>2</sup>-Cyclosporine or (Nva)<sup>2</sup>-Cyclosporine at dosages indicated above, substantial reduction in the number of animals evidencing EAV is observed compared with results for control groups.

## 2. JUVENILE DIABETES TYPE I

### BB/Worcester Rat Model

The trial is based on the general methodology described by Like et al. in Am.J.Pathol. 117, 92-97 (1984). For purposes of the 5 test groups of BB/W (0 and Q) rats (a strain spontaneously developing auto-immune diabetes of the juvenile diabetes type I) aged 60 days are employed. Test groups receive test substance, i.e. dihydro-(Val)<sup>2</sup>-Cyclosporine or (Nva)<sup>2</sup>-Cyclosporine, at a dosage of from 5 to 25 mg/kg/day p.o. for 10 days. Control groups 10 receive olive oil only in place of test substance. All test animals are screened regularly for the occurrence glycosuria.

On administration of dihydro-(Val)<sup>2</sup>-Cyclosporine or (Nva)<sup>2</sup>-Cyclosporine at the above indicated dosage rates, marked reduction in the number of rats exhibiting diabetes, e.g. exhibiting abnormally elevated blood glucose levels/glycosuria is observed compared with controls.

### 3. MYASTHENIA GRAVIS

#### Experimental Allergic Myasthenia Gravis (EAMG)

Testing is carried out in accordance with the general methodology described by V.A. Lennon et al. in J. Exp. Med. 141, 1365 - 1375 (1975). EAMG is induced in groups of 8 to 12 Lewis rats (Q) weighing ca. 150 - 200 g by intracutaneous injection of a mixture containing 1 part Freund's complete adjuvant (Difco, 0638-60) and 1 part of a solution comprising 10 µg purified acetylcholine receptor (obtained from Torpedo californica rats). Additional ad-  
10 juvant (pertussis,  $1 \times 10^{10}$  units) is administered s.c. into the hind paw pad. Autoantibody titres are determined at weekly intervals using ELISA technique.

#### 3.1 Prophylactic activity:

On administration of (Nva)<sup>2</sup>-Cyclosporine at a dosage of from 25  
15 to 50 mg/kg/day, p.o. 5 days a week commencing on the day of immunisation, inhibition of antibody formation compared with controls receiving placebo is observed.

#### 3.2 Therapeutic activity:

On administration of (Nva)<sup>2</sup>-Cyclosporine at dosages of from 25 to  
20 50 mg/kg/day p.o. 5 days a week and commencing after autoantibodies have already been formed (generally ca. 14 days after immunisation) significant reduction in autoantibody titre is observed compared with control groups receiving placebo.

#### 4. MULTIPLE SCLEROSIS

##### 4.1 Preventive Activity in Experimental Allergic Encephalomyelitis (EAE)

Testing is carried out in accordance with the general methodology described by Borel et al. in Agents and Action, 6, 468 (1976). EAE is induced in groups of 8 to 12 Wistar (♂) or Lewis (♂) rats each weighing 150 to 200 g by intradermal injection into each hind foot pad of 0.1 ml of an emulsion comprising 2.5 g bovine spinal cord (lyophilised and reconstituted with 12 ml H<sub>2</sub>O), 1.5 ml Arlachel A, 8.0 ml Nujol and 0.2 ml saline containing 20 mg killed, dried Mycobacterium phlei. (Nva)<sup>2</sup>-Cyclosporine is administered at a dosage of from 25 to 50 mg/kg/day p.o. 5 days a week, commencing on the day of sensitisation and continuing for 3 weeks. Onset of EAE in control groups receiving no medication generally commences between 9 and 16 days after sensitisation and is marked by symptoms of paralysis in the hind limbs and tail. Test animals are examined daily for the symptoms of the disease and disease occurrence is scored as positive when complete involvement of both hind legs and the tail is observed. The test animals are kept under observation for a total period of 25 days.

On administration of (Nva)<sup>2</sup>-Cyclosporine at the above indicated dosage rates substantial reduction of occurrence of EAE is observed over the test period in comparison with occurrence in control groups receiving placebo.

#### 4.2 Activity in Established EAE

Testing is carried out analogously to 4.1, but with administration of (Nva)<sup>2</sup>-Cyclosporine commencing on day 8 to day 9 after sensitisation (i.e. immediately prior to appearance of disease symptoms), at a dosage of from 25 to 50 mg/kg p.o. administered daily or every 2nd day, and continuing for ca. 14 days. During the treatment period animals are examined daily for symptoms of the disease, and scored as under 4.1.

On administration of (Nva)<sup>2</sup>-Cyclosporine at the above indicated dosage rates substantial reduction of appearance of EAE disease symptoms is observed over the test period in comparison with appearance in control groups receiving placebo.

## 5. SYSTEMIC LUPUS ERYTHEMATOSUS

### (NZB/NZW)F1 mouse model:

Trials are based on the (NZB/NZW)F1 mouse strain as described and discussed by Steinberg et al. in Bulletin on the Rheumatic Diseases 28, nos. 4-5, 940 - 946 (1977-78) published by The Arthritis Foundation, Atlanta, Georgia. Females of this strain spontaneously develop characteristics of the SLE syndrome including formation of anti-DNA and anti-erythrocyte autoantibodies as well as proteinuria at age ca. 5 to 7.5 months. The condition ultimately leads to death.

For the purpose of the trial groups of 6 to 8 ♀ mice are employed. Treatment with (Nva)<sup>2</sup>-Cyclosporine at dosages of from 50 to 100 mg/kg/day p.o. administered 5x weekly and continuing for ca. 8 to 10 weeks commences i) prior to spontaneous development of autoantibodies, e.g. at ca. 5 months' age and ii) subsequent to spontaneous development of autoantibodies, e.g. at ca. 8 - 9 months of age. Anti-DNA and anti-erythrocyte antibody titres are measured at regular intervals during the trial period employing ELISA technique and during the trial period commencing from ca. 120 week prior to (Nva)<sup>2</sup>-Cyclosporine administration. Additional parameters subject to control are development of proteinuria (measured 1x/week) and life span. Results in groups treated as under (i) and (ii) above indicate prophylactic and therapeutic effectiveness respectively.

25 On administration of (Nva)<sup>2</sup>-Cyclosporine at the above indicated dosage levels, substantial reduction of autoantibody titres and occurrence of proteinuria as well as an increase in average life span is observed in both prophylactic and therapeutic treatment

regimens as compared with results for control groups receiving placebo.

## 6. HAEMOLYTIC ANAEMIA

### Rat Erythrocyte Induced Anti-Mouse Erythrocyte Autoantibody

#### 5 Model

Testing is carried out in accordance with the general methodology described by Naysmith et al. in Immunological Rev. 55, 54 - 86 (1981). Four injections each comprising ca.  $10^8$  well-washed rat erythrocytes are administered to groups of young, normal/healthy mice at days 0, 7, 14 and 21 of the test, injection being effected i.p.. From day 21 on the animals are bled at fixed 5 to 7 day intervals into citrate saline, and a direct Coomb's test is performed using a broad spectrum sheep anti-mouse immunoglobulin antiserum. Positive reaction in the Coomb's test is generally observed in control animals receiving no medication from ca. week 3 to 4 of the trial on. The trial is continued for ca. 10 to 12 weeks.

#### 6.1 Prophylactic activity

On administration of (Nva)<sup>2</sup>-Cyclosporine at a dosage of from 50 to 100 mg/kg/day p.o. administered 5 days a week commencing on day 0 of the trial and continuing for ca. 4 to 6 weeks, marked reduction in the number of animals reacting positively in the Coomb's test up until trial completion is observed compared with control groups receiving olive oil only.

## 6.2 Therapeutic activity

On administration of (Nva)<sup>2</sup>-Cyclosporine at dosages of from 50 to 100 mg/kg/day, p.o. 5 days a week commencing after autoantibodies have already been formed (positive reaction in Coomb's test, 5 generally ca. 21 to 28 days after day 0) and continuing for 4 to 6 weeks, significant reduction of response in the Coomb's test is observed as compared with control groups receiving olive oil only.

## 7. GLOMERULONEPHRITIS

### 10 Back-Ground

Female NZB/W hybrid mice spontaneously develop renal disease characterised by immunoglobulin (Ig) and complement (C') deposition at 3 to 6 months of age with histological glomerulonephritis and proteinuria from about 6 months and thus provide an appropriate animal model for the disease glomerulonephritis as it occurs in man.

### Method

Female NZB/W mice are randomly sorted into control and treatment groups of 10 mice each. Each mouse is ear-marked to permit individual identification. Experiments are started when the mice are aged 12, 24 or 36 weeks old and treatment continues for 12 weeks. (Nva)<sup>2</sup>-Cyclosporine is administered at dosages of from 50 to 100 mg/kg given orally by gavage five times a week.

The degree of proteinuria is estimated by staining 10  $\mu$ l urine spots on filter paper with bromophenol blue. A thin-layer-chromatography scanner connected to an integrating computer is used to quantify the intensity of the stained urine spots compared to a 5 serial dilution of bovine serum albumin. Levels of protein above 100 mg% are considered abnormal and positive.

Mice are sacrificed at the end of each experiment and the kidneys routinely prepared for histological examination. Direct immunofluorescent studies are performed using antisera directed against 10 mouse Ig and C3 (Nordic). Glomerulonephritis is classified into (i) mild endothelialmesangial lesions, (ii) more severe segmental proliferative, and (iii) most severe membrano-proliferative type. Scoring is from no lesion progressing to severe lesion. Immunofluorescent scoring ranges from nothing to strong. Results 15 are compared with a control group receiving placebo (olive oil) only. If an animal dies while having proteinuria during the experiment, it is considered positive.

On administration of (Nva)<sup>2</sup>-Cyclosporine at dosages indicated above marked reduction of deposition of immunoreactants and 20 histological evidence of glomerulonephritis is observed as compared with control groups receiving placebo only.

As previously indicated effectiveness of dihydro-(Val)<sup>2</sup>-Cyclosporine and (Nva)<sup>2</sup>-Cyclosporine in accordance with the present invention may also be demonstrated in clinical trials, for example in the case of treatment of Juvenile diabetes type 1, conducted as follows:

The trial is carried out employing volunteer, insulin-dependent diabetics, diagnosed as exhibiting Juvenile Diabetes Type I. Diagnosis is made on clinical grounds in non-obese subjects with confirmed hyperlycaemia [National Diabetes Data Group, Diabetes 28, 1039 (1979)]. All subjects have a serum immuno-reactive C-peptide concentration within the normal fasting range, tested in the fasting state with and without glucagon stimulation (0.2 p mole/ml), and have received insulin therapy for less than 12 months.

15 Subjects are admitted to hospital for an average period of ca. 5 days at the beginning of the trial. Baseline creatinine clearance, urinalysis, serum creatinine, blood urea nitrogen, serum glutamic-oxaloacetic transaminase (SGOT), and alkaline phosphatase levels are determined to ensure that these are normal. Oral 20 administration of test substance, i.e. dihydro-(Val)<sup>2</sup>-Cyclosporine or (Nva)<sup>2</sup>-Cyclosporine is commenced at dosages of from ca. 5 to 25 mg/kg/day/patient generally administered once or in divided dosages each 12 hours. Daily serum concentrations are measured by radioimmunoassay and individual dosage rates adjusted to maintain 25 blood concentrations of from ca. 200 to 2,500 ng/ml at 12 hours. After discharge patients are seen weekly for 2 weeks and monthly thereafter. At each in-clinic visit blood is drawn for determination of test substance levels, creatinine and electrolyte concentrations and for hematological and liver function tests; basal 30 and glucagon-stimulated plasma C-peptide concentrations are measured at 1 month and at 3 month intervals thereafter.

During the course of the trial all patients are treated with purified pork insulin (Iletin II) and are encouraged to effect administration 2x daily and to monitor blood glucose concentrations using visual matching or reference meter methods with 5 reagent strips. They are instructed in a diabetic diet appropriate for maintenance of normal body weight and activity. Insulin dosage is adjusted as far as possible to achieve a mean blood glucose level of 7.8 m mole/litre before the main meals and evening snack. Insulin dosage is reduced when control of glycaemia is consistent with these targets or in order to avoid hypoglycaemia. Subjects for which insulin is completely withdrawn for at least 1 week without loss of target control or development or ketonuria and who need not resume insulin treatment during the course of the study are classed as no longer-insulin-requiring 15 (NIR). Where subjects becoming NIR subsequently develop hyperglycaemia exceeding treatment goals, application of the oral hypoglycemic agent glybenclamide is undertaken.

Results indicate a marked and significant increase of NIR remission in subjects participating in the trial as compared with 20 remissions (i.e. spontaneous NIR remissions) recorded for groups of juvenile diabetics for whom no therapy is attempted or in comparison with NIR remissions recorded in groups of juvenile diabetics receiving alternative intervention therapy. Furthermore results would indicate that therapy is markedly well tolerated, 25 e.g. as evidenced by measurements for other physiological parameters measured during the course of the trial.

In accordance with the foregoing the present invention provides: a method for the treatment of an autoimmune disease selected from the group consisting of: a) Uveitis; b) Juvenile diabetes type I; c) Myasthenia gravis; d) Multiple sclerosis; e) Systemic lupus erythematosus; f) Haemolytic anaemia, and g) Glomerulonephritis; in a subject in need of such treatment, which method comprises administering to said subject an effective amount of (Nva)<sup>2</sup>-Cyclosporine or, in the case of disease a) or b) above, dihydro-(Val)<sup>2</sup>-Cyclosporine.

10 In an alternative embodiment the present invention also provides: the use of (Nva)<sup>2</sup>-Cyclosporine or dihydro-(Val)<sup>2</sup>-Cyclosporine for the treatment of an autoimmune disease selected from the group consisting of: a) Uveitis and b) Juvenile diabetes type I, or use of (Nva)<sup>2</sup>-Cyclosporine for the treatment of an autoimmune disease  
15 selected from the group consisting of: c) Myasthenia gravis, d) Multiple sclerosis, e) Systemic lupus erythematosus, f) Haemolytic anaemia, and g) Glomerulonephritis.

In a yet further alternative embodiment the present invention also provides: the use of (Nva)<sup>2</sup>-Cyclosporine or of dihydro-  
20 (Val)<sup>2</sup>-Cyclosporine for the manufacture of a pharmaceutical composition for the treatment of an autoimmune disease selected from the group consisting of: a) Uveitis, and b) Juvenile diabetes type I, or use of (Nva)<sup>2</sup>-Cyclosporine for the manufacture of a pharmaceutical composition for the treatment of an autoimmune  
25 disease selected from the group consisting of: c) Myasthenia gravis, d) Multiple sclerosis, e) Systemic lupus erythematosus, f) Haemolytic anaemia, and g) Glomerulonephritis.

By "treatment" as used in the above definitions is to be understood both curative treatment as well as prophylactic treatment

where appropriate, e.g. as indicated in the above described animal model test methods.

Doses for clinical use in accordance with the method of the invention will of course vary depending upon, e.g. the mode of administration, the particular autoimmune disease or the specific condition of the subject to be treated and the effect desired. In addition dosaging will generally require adjustment for individual patients receiving treatment in order to establish an appropriate long-term drug serum concentration, e.g. by administration of an initial daily starting or "loading" dose with subsequent dose adjustment (generally reduction) in accordance with achieved serum levels as determined, e.g. by regular RIA monitoring. However in general satisfactory results are obtained on administration of both dihydro-(Val)<sup>2</sup>-Cyclosporine and (Nva)<sup>2</sup>-Cyclosporine in a dose range of from about 3 to about 30, e.g. from about 10 to about 25 mg/kg body weight/day administered to the patient orally once or, in divided doses, 2 or 3x a day. Where i.v. administration is indicated, for example in the initial phase of treatment, lower dosages, e.g. of the order from about 1 to about 10, e.g. ca. 3 to 5 mg/kg/day are generally indicated. A suitable oral daily dosage, e.g. for adult patients, is accordingly of the order of from about 225 to about 2,000, e.g. from about 750 to about 1,800 mg/day, and unit dosage forms for oral administration suitably comprise from about 75 to about 2,000, e.g. from about 250 to about 1,500 mg dihydro-(Val)<sup>2</sup>-Cyclosporine or (Nva)<sup>2</sup>-Cyclosporine/dose, together with a pharmaceutically acceptable diluent or carrier therefor.

Dihydro-(Val)<sup>2</sup>-Cyclosporine is well tolerated at dosages contemplated for use in accordance with the present invention. Thus 26 week toxicity trials in the rat and beagle dog indicate a no toxicity dosage level of  $\leq 8$  and 32 mg/kg/day respectively.

(Nva)<sup>2</sup>-Cyclosporine is also tolerated at dosages contemplated for use in accordance with the present invention. Indeed it has very surprisingly been found that while (Nva)<sup>2</sup>-Cyclosporine generally possesses a high order of immunosuppressive activity, and may be shown to possess particularly advantageous properties in relation to immunosuppressive use in accordance with the present invention, e.g. as hereinbefore indicated, it is unexpectedly lacking in side effects, in particular hepato- and nephro-toxic effects, hitherto experienced employing Cyclosporine as medication. This relative freedom from undesirable side effects may for example be demonstrated in standard short-term toxicity trials.

One such trial is carried out employing 3 groups of 5 HAN Wistar rats. Group 1 receives 100 mg/kg (Nva)<sup>2</sup>-Cyclosporine/day, Group 2 receives 100 mg/kg Cyclosporine/day and Group 3 (control) remains untreated. Both (Nva)<sup>2</sup>-Cyclosporine and Cyclosporine are administered in solution in olive oil by means of a stomach tube. The following parameters are evaluated: body weight; mortality; serum and urine analysis; kidney and liver analysis, macroscopy and histology (urine volume, kidney weight, creatine clearance, Na and K levels, SGPT and SGOT levels etc); and neurological symptoms. Results obtained for neurological symptoms, mortality, kidney biochemistry and histology (in particular swelling/regeneration of tubular epithelial cells and tubular necrosis) and liver biochemistry in Group 1 are in all cases directly comparable with results obtained for control Group 3 and show overall marked superiority in comparison with results obtained for Group 2. No adverse reaction in Group 1 as compared with Group 2 is observed in respect of other parameters measured.

Suitable galenic formulations for the administration of cyclosporins, including the current commercially available Cyclosporine

drink solution, are described and claimed in DOS 29 07 460 (= Japanese patent application No. 27228/1979, U.K. patent specification No. 2 015 339 B and US patent No. 4,388,307). Pharmaceutical compositions for use in accordance with the present invention may be prepared directly analogously to the methods disclosed therein, employing dihydro-(Val)<sup>2</sup>-Cyclosporine or (Nva)<sup>2</sup>-Cyclosporine as active ingredient. Liquid oral compositions comprising both dihydro-(Val)<sup>2</sup>-Cyclosporine and (Nva)<sup>2</sup>-Cyclosporine (e.g. drink solutions), as the Cyclosporine drink solution  
10 itself, are suitably administered together with a chocolate flavouring agent, e.g. as described in Example 1 of the aforementioned US patent No. 4,388,307.

The following are examples for the preparation of liquid pharmaceutical preparations suitable for the administration of  
15 dihydro-(Val)<sup>2</sup>-Cyclosporine and (Nva)<sup>2</sup>-Cyclosporine. All percentages are by weight.

EXAMPLE 1: DIHYDRO (VAL)<sup>2</sup>-CYCLOSPORINE DRINK SOLUTION

<u>COMPONENT</u>	<u>CONTENT</u>
i) Dihydro-(Val) <sup>2</sup> -Cyclosporine	5 - 10 % , e.g. 7.0 %
ii) Ethanol (absolute)	10 - 12 % , e.g. 10.5 %
5 iii) Cremophor <sup>®</sup> RH 40	ca. 4 % , e.g. 2.8 %
iv) Maisine <sup>®</sup>	30 - 40 % , e.g. 65.0 %
v) Labrafil <sup>®</sup> 2125	to 100 % total

[Cremophor<sup>®</sup> RH 40 = reaction product of hydrogenated castor oil and ethylene oxide in a molar ratio of ca. 1:40 - available from BASF AG, Ludwigshafen DT. Maisine<sup>®</sup> = trans-esterified maize oil - available from ETS. Gattefossé, Boulogne-Brillancourt FR. Labrafil<sup>®</sup> 2125 = polyoxyethylated kernel oil - available from ETS. Gattefossé, Boulogne-Brillancourt FR.]

The desired quantity of i) is dissolved in components ii) through 15 iv) in conventional manner, the solution brought to an end-volume of 100 % by addition of v) and the resultant mixture filled into a small vial. For the purposes of administration, the solution is conveniently mixed into a flavour-masking composition, e.g. into chocolate-flavoured milk, prior to administration.

EXAMPLE 2: DIHYDRO-(VAL)<sup>2</sup>-CYCLOSPORINE SOFT GELATINE CAPSULES

<u>COMPONENT</u>	<u>CONTENT</u>
i) Dihydro-(Val) <sup>2</sup> -Cyclosporine	15 - 25 %
ii) Ethanol (absolute)	2 - 5 %
5 iii) Imwitor <sup>®</sup> 742	10 - 40 %
iv) Maisine	40 - 60 %

[Imwitor<sup>®</sup> 742 = glycerine-ester - available from Dynamite Nobel AG, Troisdorf-Oberlar SN].

The quantity of i) for a single dosage is dissolved in components  
10 ii) to iv) using conventional techniques to give a solution suitable for filling into a soft gelatine capsule.

EXAMPLE 3: (NVA)<sup>2</sup>-CYCLOSPORINE DRINK SOLUTION

<u>COMPONENT</u>	<u>CONTENT mg/ml</u>
i) (Nva) <sup>2</sup> -Cyclosporine	100.0
15 ii) Ethanol (absolute)	150.0
iii) Labrafil <sup>®</sup> 2125	350.0
iv) Corn oil	to a total of 922.0 (≅ 1 ml)

The individual components are compounded as in example 1 and the  
obtained solution filled into individual containers in an amount  
20 giving the required dosage of i). The solution is preferably  
ingested undiluted.

CLAIMS

1. A method for the treatment of an autoimmune disease selected from the group consisting of:

a) Uveitis; b) Juvenile diabetes type I, c) Myasthenia gravis, d) Multiple sclerosis, e) Systemic lupus erythematosus, f) Haemolytic anaemia, and g) Glomerulonephritis;  
in a subject in need of such treatment, which method comprises administering to said subject an effective amount of (Nva)<sup>2</sup>-Cyclosporine or, in the case of disease a) or b) above, dihydro-10 (Val)<sup>2</sup>-Cyclosporine.

2. The use of (Nva)<sup>2</sup>-Cyclosporine or of dihydro-(Val)<sup>2</sup>-Cyclosporine for the treatment of an autoimmune disease selected from the group consisting of:

a) Uveitis and b) Juvenile diabetes type I,  
15 or use of (Nva)<sup>2</sup>-Cyclosporine for the treatment of an autoimmune disease selected from the group consisting of:  
c) Myasthenia gravis, d) Multiple sclerosis, e) Systemic lupus erythematosus, f) Haemolytic anaemia, and g) Glomerulonephritis.

3. The use of (Nva)<sup>2</sup>-Cyclosporine or of dihydro-(Val)<sup>2</sup>-Cyclosporine for the manufacture of a pharmaceutical composition for  
20 the treatment of an autoimmune disease selected from the group consisting of:

a) Uveitis, and b) Juvenile diabetes type I,  
or use of (Nva)<sup>2</sup>-Cyclosporine for the manufacture of a pharmaceutical composition for the treatment of an autoimmune disease  
25 selected from the group consisting of:

c) Myasthenia gravis, d) Multiple sclerosis, e) Systemic lupus erythematosus, f) Haemolytic anaemia, and g) Glomerulonephritis.